

# MEASUREMENT OF MICROSCALE BIO-THERMAL RESPONSES BY MEANS OF A MICRO-THERMOCOUPLE PROBE

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**Abstract-** We developed a micro-thermocouple probe in order to measure the thermal responses of a cell. A micropipette with a tip 1  $\mu\text{m}$  in diameter was used for the base of the probe. A Pt/Au junction was constructed on the tip. The average thermoelectric power of the probe was 2.1  $\mu\text{V/K}$ . The temperature and time resolutions of the probe were investigated by using a laser beam to irradiate the tip.

**Keywords-** Thermocouple, microscale, bio-thermal response, micropipette, cell

## I. INTRODUCTION

Measurements of thermal rapid responses in a biological micro-region, particularly a cell, can provide new physiological information. It is known that cellular temperature change, that is, heat production, is caused by the reaction to the administration of a certain substance to brown adipocytes or to the electrostimulation of neurons. However, a real-time measurement technique on a target of a cell has not been established. Measurement using infrared imaging microscopy [1] has the advantage of non-contact, but the temperature and spatial resolutions are lower than the required values. The method using temperature-dependent fluorescence [2] has problems with temperature resolution and biological toxicity. With the recent development of micro/nano fabrication techniques, contact measurement by means of a sensor probe such as a thermocouple has expanded the capabilities of microscale temperature measurement. Fish et al. [3] made a thermocouple probe based on a micropipette. There are also several reports on the development of the thermocouple probe based on the atomic force microscope (AFM) cantilever probe [4, 5]. A micropipette is suitable for application in cellular measurement because a micropipette (glass capillary) has been used for cell operation and the injection of DNA fragments or substances into a cell. Furthermore, micropipettes also have been used to measure the electric potential at the cell membrane as a patch electrode with the tip improved for contact with the cell membrane [6]. Hence, we adopted a micropipette as the base of a thermocouple probe. The aim of this study was to develop a micro-thermocouple probe and to apply it to the measurement of thermal responses of a cell. We describe here the thermoelectric characteristics of the developed thermocouple probe and the capability of measuring thermal responses at the cellular level.

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## II. FABRICATION OF THERMOCOUPLE PROBE

The basic steps in fabricating a micro-thermocouple probe (Fig. 1) were as follows. (1) A glass micropipette, 1  $\mu\text{m}$  in external diameter and 50 mm in length, was made from a glass tube, 1 mm in external diameter, using a pipette puller (PB-7, Narishige). This puller partially heated a glass tube with a hot wire and drew it with weights. The size of the tip depended on the heating temperature and the weight. (2) A thin platinum (Pt) film 30 nm thick was deposited on the micropipette by means of the ion-sputtering technique. Before the Pt was deposited, a thin chrome film 10 nm thick was deposited on the pipette for good adhesion between the Pt and the glass. (3) A silane coupler (VM-652, HD MicroSystems) was applied as a primer for good adhesion of the polyimide coating (Pyralin® PI2556, HD MicroSystems), which was used as an insulating layer. We also used  $\text{SiO}_2$  instead of polyimide. (4) A gold (Au) thin film was deposited by means of the ion-sputtering technique. (5) A coating of polyimide/ $\text{SiO}_2$  was applied. (6) Finally, a coating of MPC (2-methacryloyloxyethyl phosphorylcholine) copolymers was put on the tip for good biocompatibility. MPC copolymers have an affinity for phospholipids due to the phosphorylcholine polar groups on the MPC copolymer surface [7].

The junction of the Pt and Au was created by the

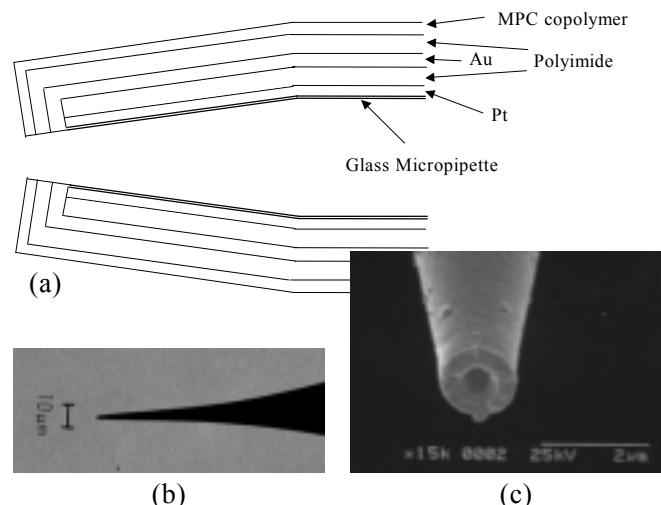


Fig.1. (a) Schematic representation of the structure of the thermocouple probe based on a micropipette. (b) Microscopy image. (c) Scanning electron micrograph of the tip.

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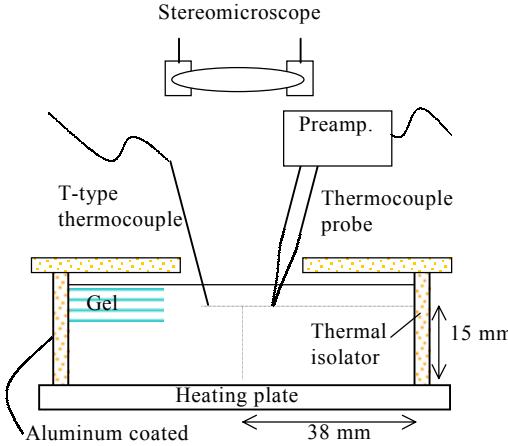


Fig.2. Experimental apparatus for the measurement of thermoelectric power.

following two methods. First, when the probe on which the liquid polyimide was dip-coated was held upright, it was found that the Pt surface was bared at the tip because of surface tension and gravity. Second, after the polyimide film was formed, an N<sub>2</sub>-dye-laser beam (390nm, max 22  $\mu$ J/pulse, 0.5  $\mu$ m in spot diameter, LaserScissors<sup>TM</sup>, Cell Robotics) directly heated the tip, and the polyimide film within 1  $\mu$ m in diameter was ablated. In both methods, whether Pt surface was bared could be judged by measuring the leakage current of the probe immersed in an electrolytic solution. However, with both methods, it was difficult to precisely measure the bared areas. They could be estimated by such techniques as scanning electron microscopy, energy dispersive X-ray spectrometry, or electrical methods. Although there was a problem with the junction to be investigated, we initially used the first probe and investigated its characteristic features of thermoelectricity.

### III. THERMOELECTRIC CHARACTERISTICS

#### A. Impedance

Resistance of the probe was 347  $\Omega$  (S.D.=72 $\Omega$ , test voltage: 20mV,  $n$  (probes)=16). The factors affecting this value could have been the thickness of the Pt and Au film and the junction size. Considering the thickness of the polyimide film between Pt and Au, the probe had been expected to have large capacity reactance, but the reactance at 100kHz was -3  $\Omega$  (SD=2  $\Omega$ , 20mV,  $n$ =16).

#### B. Thermoelectric Power

The thermoelectric power of each probe was measured by using the calibration system shown in Fig. 2. Since the thermoelectric power was slight, a preamplifier consisting of a non-inverting negative feedback amplifier circuit and

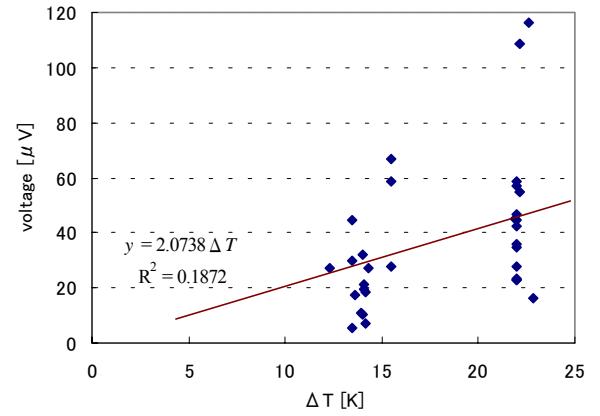


Fig.3. A plot of the thermoelectric power of each probe.  $N=32$  (16 probes were investigated).

a low-pass filter circuit was located near the probe. The signals occurring after the preamplifier were amplified by the main amplifier (AD-641G, Nihon Koden). The tip of the probe was immersed into a temperature-controlled bath, and its thermoelectric power was recorded. The reference temperature was recorded by a T-type small thermocouple (200 $\mu$ m in diameter) located at the point of symmetry to the tip of the probe as viewed through a stereomicroscope. Figure 3 shows the thermoelectric power in  $\mu$ V of each probe with temperature differences of 14 K and 22 K between the junction and the reference point,  $\Delta T$ . The results of 16 probes are plotted. The linearization of these data indicated that the average thermoelectric power was 2.1  $\mu$ V/K (max 5.0  $\mu$ V/K). It is known that the thermoelectric power of Pt/Au thin films is less than that of bulk Pt/Au (7.2 $\mu$ V/K). However, the reason for the low thermoelectric power seemed to be that the conditions of the Pt and Au thin films and their junction did not satisfy the quality requirements. For the same reason, there was a variety of these thermoelectric powers among the probes. In addition, a significant correlation between the resistance and the thermoelectric power of each probe was not obtained.

#### C. Temperature and Time Resolutions

In order to evaluate the temperature and time resolutions of the probe, the temperature responses caused by heating the tip with a laser beam were investigated. In the beginning, the irradiation time of the beam from the laser diode (650 nm $\pm$ 3%, max 5mW) was controlled by an electromagnetic shutter (1/15 sec), and the beam was condensed through the lens. Although the received energy at the tip could have been estimated from the laser output, the irradiation time, and the received area, we identified how much the temperature rose from the known thermoelectric power. The average peak voltage was

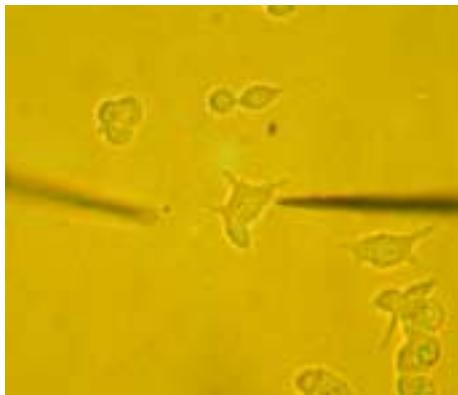


Fig. 4. Approach of the thermocouple probe to a PC12 cell. Right: The thermocouple probe. Left: Micropipette for administration of a substance.

equivalent to an increase of 0.25 K, which was in accordance with the thermoelectric power. Comparing the peak with the amplitude of the remaining low-frequency noise, it was obvious that the identification limit in temperature was lower than 0.25 K. In this measurement system, the time resolution cannot be predicted accurately because the irradiation time is rather long and the received area of the tip cannot be controlled.

#### IV. DISCUSSION

The advantages of using a micropipette as the base of the thermocouple are as follows. (1) A micro-tip has already been formed; (2) the size and form of the tip can be changed in making the micropipette; (3) various metals containing alloys, which can be coated by ion sputtering or vacuum evaporation, can be selected; (4) the injection function is combined. Furthermore, considering the multi-functionalities of the glass capillary, photo stimulation and measurement of an optic fiber [8] and strain gauge [9] can be combined.

Using Pt and Au has the following advantages: (1) the thermal expansivity is similar to that of glass; (2) they are unreactive; and 3) they are treatable as ion-sputtering targets. For modifying temperature sensitivity, however, it is effective to use a pair of metals whose thermoelectric power is larger than that of Pt/Au. Since it is difficult to apply photolithography to a 3-D probe such as a micropipette, in this study, we were obliged to use the passive method of controlling the junction. In addition to ablating the polyimide by laser beam, another technique such as ion-beam etching should be used in future studies.

While improving the probe, it is necessary to reduce the noise of the measurement system and to improve S/N by an effective signal processing. One must especially take into account the fact that the thermal conductivity of liquid (culture) is 27 times larger than that of air and it is difficult to measure thermal changes in liquid. However,

it is possible to make a micropipette penetrate or attach to a cell membrane and to enhance thermal conduction to the probe.

The developed thermocouple probe is currently applied to the measurement of the thermal responses of a cell (Fig. 4). Our interest is focused on the heat production of various cells to cytokines, nerve transmitter substances, or antibiotic substances.

#### V. CONCLUSION

We developed a micro-thermocouple probe in order to measure the thermal responses of a cell. A micropipette with a tip 1  $\mu$ m in diameter was used for the base of the probe. A Pt/Au junction was constructed on the tip. The result of calibration with a temperature-controlled bath demonstrated that the average thermoelectric power was 2.1  $\mu$ V/K. Voltage changes caused by heating the tip with a laser beam were measured in order to evaluate the temperature and time resolutions. The results indicated that temperature changes of less than 0.25 K could be detected. In the future, we would like to improve the thermoelectric performance of the probe and apply it to the measurement of the thermal response of a cell.

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